



The Effect of Bacteria Penetration on Chalk Permeability

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Introduction

Numerous mechanisms have been proposed in the literature through which microorganism can be used for enhanced oil recovery. One of them is the changes in flow behavior due to bioclogging or selective plugging (Afrapoli et al., 2011; Armstrong and Wildenschild, 2012). Injection of bacterial populations may serve plugging water-bearing zones and attaining more uniform displacement of oil from heterogeneous rock (Li et al., 2012).

Study of bacteria penetration is important to understand how deep/far bacteria can travel through the porous media, plug the water-bearing zones of a reservoir, and thus altering the flow paths and improving sweep efficiency. Although previous investigations have revealed that bacteria can penetrate deeply into porous media (Jenneman et al., 1985; Kalish et al., 1964), a complete understanding of the penetration behavior of bacteria is lacking. Furthermore, there is limited amount of publications on microbial penetration/plugging study on chalk as earlier studies were conducted on sandstones (Jenneman et al., 1985; Kalish et al., 1964), micromodels (Afrapoli et al., 2011), glassbeads (Armstrong and Wildenschild, 2012) and sandpacks (Sarkar et al., 1994; Weiss et al., 1995). In all these porous media the pore sizes are considerably larger than in chalk rocks.

This study focused on core flooding experiment to investigate bacteria penetration in low permeable chalk and its effect on permeability. The porous media used were Stevn Klint outcrop samples with low permeability, ranging from 2 mD to 4 mD. Two bacteria species, *Bacillus licheniformis* 421 and *Pseudomonas putida* K12, were used to represent spore forming and non-spore forming bacteria.

Experimental Work

Core flooding experiments

Each individual core was cleaned by flooding with toluene and absolute ethanol to remove any organic material inside the core. After cleaning, the core was dried in the oven at 80°C overnight. The dry weight and wet weight were measured in an analytical balance to calculate core porosity. The dry core was assembled in sterile Hassler core holder. Approximately 7 pore volumes injected (PVI) of 75% ethanol were injected and the 75% ethanol saturated core was left inside the core holder overnight in order to sterilize the core plug. Subsequently, the 75% ethanol was displaced by injection of 7PVI of sterile MQ water. The core was then injected with bacteria inoculum in sterile synthetic seawater (SS) media supplemented with 5mM NaNO₃ and 4% molasses. The bacteria injections under different concentrations were conducted to investigate the effect of plugging on core permeability. The effluent was collected every PV for bacteria enumeration and cell observation. Injection pressure and pressure difference were monitored throughout these processes. The recorded pressure difference during MQ flooding and bacterial flooding was used to calculate core permeability using Darcy's Law.

Inoculum preparation, bacteria enumeration and observation

The bacteria inoculum was grown in the enrichment agar media for 24 hours then diluted with 0.85% NaCl. The optical density (OD) of the bacteria solution was adjusted to the desired value at the wave length of 600nm using a spectrophotometer. Approximately 10% (v/v) bacteria solution was inoculated into SS media for core flooding experiment. The bacteria and SS media were homogenized by a vortex for 3 minutes. The collected effluents were used for bacteria enumeration by plate count method. The remaining effluents after the plate count were fixed with 2% (v/v) formaldehyde for 30 minutes, stained with 0.5% (v/v) 4',6-diamidino-2-phenylindole (DAPI) and observed under fluorescence microscope.

Result and Discussion

A summary of the initial conditions of the cores and bacteria inoculum concentrations used for the experiments is presented in Table 1. One of the major concerns for MEOR application in chalk formations is whether the bacteria can penetrate deeply into formations as the pore throat sizes are

almost comparable with the sizes of bacteria vegetative cells. David and Updegraff (Davis and Updegraff, 1954) mentioned that the pore throat sizes of the rock should be at least twice the cell size to allow passage of microbes through rock without serious plugging. The Stevns Klint core samples used in this study have unimodal pore throat size distribution in the range of 0.004-6.1 μm with mean value of about 0.5 μm (Tweheyo et al., 2006). The specific bacteria strains used in this study *B. licheniformis* 421 has an approximate cell size 0.5-0.75 μm in diameter and 2-4 μm in length and *P. putida* K12 has an approximate cell size 0.5 μm in diameter and 1-2 μm in length.

Our experiments showed that both *B. licheniformis* 421 and *P. putida* K12 were able to penetrate the low permeable Stevn Klint chalk. The collected effluents from the core flooding experiments showed that *B. licheniformis* 421 penetrate in form of spores, whereas the *P. putida* K12 penetrate in form of vegetative cells. The spores of *B. licheniformis* 421 grew as vegetative cells during bacteria enumeration. In addition, a higher number of *B. licheniformis* 421 was detected in the effluents as compared with *P. putida* K12 (fig.1 and fig.2). This happened even when higher inoculum of *P. putida* K12 was used for the experiment. This may indicate that survival/motion of bacteria can be mainly due to spores formations. After 12 days of incubation, more cells were detected in the effluents when additional brine flooding was conducted. The media pH was decreasing slightly during bacteria injection due to acid and/or CO_2 production. After 12 days of incubation, the pH showed increasing trend, as dissolution of Ca^{2+} took place.

Table 1. Summary of the initial conditions of the cores and bacteria inoculum concentration

Core number	Bacteria	Initial porosity (%)	Initial permeability (mD)	Bacteria inoculum (cfu/ml)
2	<i>B. licheniformis</i> 421	36.78	1.82	8.80×10^7
10	<i>B. licheniformis</i> 421	37.35	4.47	3.80×10^7
5	<i>P. putida</i> K12	38.49	4.56	3.5×10^6
9	<i>P. putida</i> K12	37.15	4.20	2.14×10^8

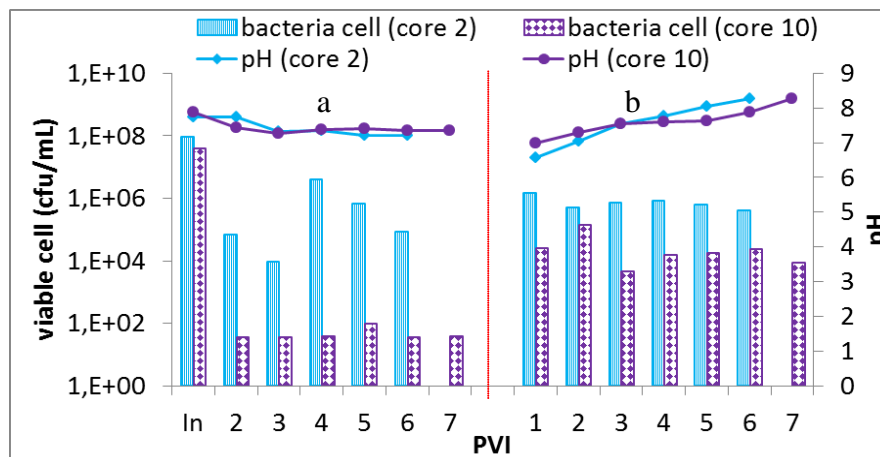


Figure 1. Viable *B. licheniformis* 421 cells detected on the effluent (a) during injection of bacteria suspension and (b) after 12 days of incubation at 50°C (core 2 and core 10). In: bacteria inoculum

The core permeability is expressed as core permeability at given time during injection (k) divided by initial permeability (k_i). Both fig. 3 and fig. 4, show core permeability is damaged continuously when bacterial population is injected for both spore forming and non-spore forming bacteria. The permeability reduction was higher for the *B. licheniformis* 421, probably due to the fact that the size was larger than the *P. putida* K12. After 12 days of incubation, permeability did not return to initial condition (k_i), even after the bacterial population was flushed by approximately 7PVI of SS. The permeability stabilized at a constant level (0.5 of k_i for *B. licheniformis* 421, 0.58 of the k_i for the low concentration of *P. putida* K12 and 0.41 of the k_i for the high concentration of *P. putida* K12).

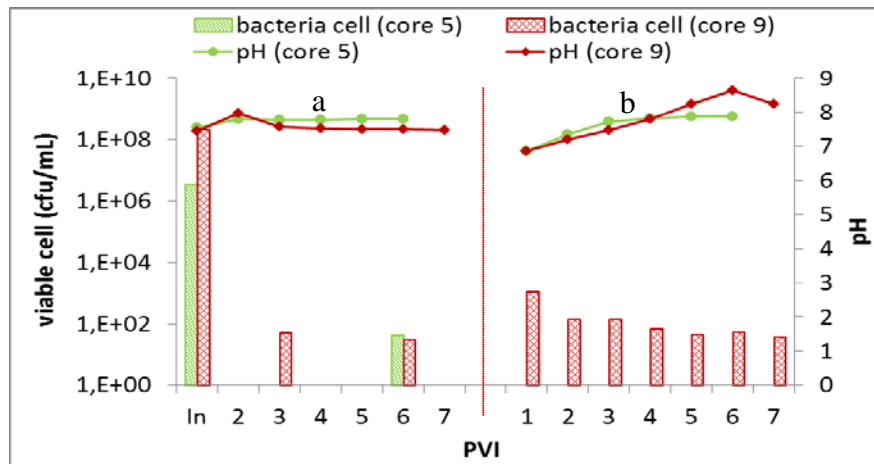


Figure 2. Viable *P.putida* K12 cells detected on the effluent (a) during injection of bacteria suspension and (b) after 12 days of incubation. Less viable cell were detected compare to *B. licheniformis* 421 injection even when higher bacteria inoculum is injected. In: bacteria inoculum

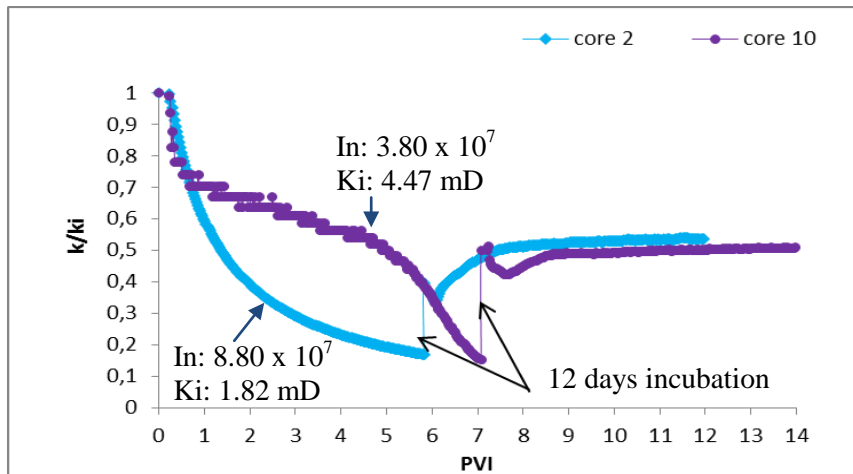


Figure 3. Permeability changes by *B. licheniformis* 421 during bacteria suspension injection and after 12 days of incubation. The core permeability was reduced continuously when bacterial population was injected and permeability did not return back when the bacterial population was flashed by brine. (In: bacteria inoculum in cfu/ml, ki : initial permeability)

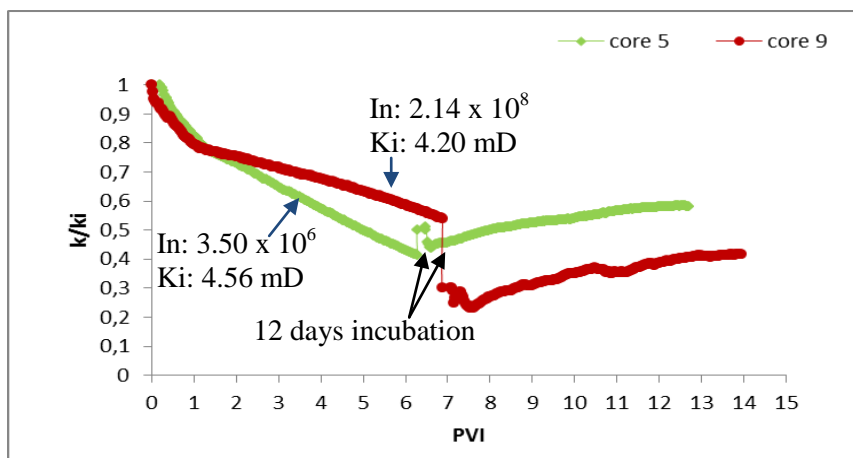


Figure 4. Permeability changes by *P.putida* K12 during bacteria suspension injection and after 12 days of incubation (starvation). The core permeability was reduced continuously when bacterial population was injected and permeability did not return back when the bacterial population was flashed by brine. (In: bacteria inoculum in cfu/ml, ki : initial permeability)

Conclusions

In this work we have shown that bacteria were able to penetrate and to be transported through the chalk porous media even though the permeability is as low as below 4 mD and the pore sizes are comparable to bacterial sizes. Bacteria cell and/or other substances (eg: extrapolymeric substances/EPS) damaged the porous medium and caused constant reduction of permeability. The spore forming bacteria have a bigger chance for survival compared to non-spore forming bacteria. In addition, a stable colony may have formed during the incubation period. When the core is flushed by injecting brine after the incubation period, the bacteria cells continue to exist (but not grow), thus the permeability did not return to initial condition.

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